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MITOSIS IN THE YEAST PHASE OF THE BASIDIOMYCETES *BENSINGTONIA YUCCICOLA* AND *STILBUM VULGARE* AND ITS PHYLOGENETIC IMPLICATIONS[1]

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Phylogenetic studies of yeasts rely on an extensive molecular and biochemical data set, but structural characters are scarce. Details of mitosis in yeasts have been studied with transmission electron microscopy and immunofluorescence. Of these two methods immunofluorescence is faster and easier and yields sufficient detail for cytological comparisons. Only three basidiomycetous yeasts have been studied thus far with immunofluorescence. Mitosis in budding cells of ascomycetous yeasts occurs in the parent, while in basidiomycetous yeasts, except in *Agaricostilbum pulcherrimum*, it occurs in the bud. Mitosis in additional yeasts in the Agaricostilbomycetidae of the Urediniomycetes was observed using immunofluorescence localization of freeze-substituted material. In *Stilbum vulgare*, mitosis occurred in the parent, but in *Bensingtonia yuccicola* it occurred in the bud as in most other basidiomycetous yeasts. *Stilbum vulgare* also had predominantly binucleate yeast cells. Nuclear small subunit rDNA sequence data showed that *A. pulcherrimum* and *S. vulgare* are more closely related to each other than to *B. yuccicola* within the Agaricostilbomycetidae. Based on the few taxa examined, mitotic and cytoskeletal characters provide phylogenetic information.

Key words: *Agaricostilbum pulcherrimum*; cytoskeleton; evolution; immunofluorescence; microtubules; nuclear small subunit rDNA; Urediniomycetes.

Knowledge about the known diversity of yeasts has rapidly expanded as their potential habitats are explored (Kurtzman and Fell, 1998; Mueller et al., 1998; Suh et al., 2003). Although generally considered ascomycetes, yeasts are widely distributed in all three classes of Basidiomycota (Fell et al., 2001), including the homobasidiomycetes (Mueller et al., 1998). Because their simplicity yields few structural characters, most data used to classify yeasts are molecular or physiological. As reviewed by McLaughlin et al. (1995), microscopic analyses of fungal cells have provided informative phylogenetic characters, such as spindle pole body form, nuclear division features, and cytoplasmic organization. Therefore, we have been exploring the mitotic and cytoskeletal patterns of basidiomycetous yeasts (Frieders and McLaughlin, 1996; McLaughlin et al., 1996) as a source of additional phylogenetic characters.

During budding in the yeast cell cycle, the products of mitosis are distributed between the parent and bud. Changes in the cytoskeleton and the positions of the nucleus and spindle are revealed more easily through immunofluorescence localization of microtubules and DNA than through traditional ultrastructural analyses. Two general mitotic patterns have been found to distinguish ascomycetous and basidiomycetous budding yeasts (McLaughlin et al., 1996). In ascomycetous yeasts, nuclear migration is associated with a discrete bundle of microtubules originating at the spindle pole body; spindle initiation and early elongation occur in the parent; and nuclear elongation proceeds from the parent into the bud (Kilmartin and Adams, 1984; Barton and Gull, 1988). In most basidiomycetous yeasts, nuclear migration is associated with a basket of cortical microtubules; spindle initiation and early elongation occur in the bud; and nuclear elongation proceeds from the bud back into the parent (Frieders and McLaughlin, 1996; McLaughlin et al., 1996).

Of the budding basidiomycetous yeasts studied cytologically, the majority are classified within the Urediniomycetes (Swann et al., 2001). Only three basidiomycetous yeasts were studied previously with immunofluorescence (Frieders and McLaughlin, 1996; McLaughlin et al., 1996), and these three taxa are placed within the three major clades of this class. *Kriegeria eriophori* and *Septobasidium carestianum* both share the common basidiomycetous yeast mitotic pattern (McLaughlin et al., 1996). *Agaricostilbum pulcherrimum*, however, possesses characters intermediate between those of ascomycetes and basidiomycetes (Frieders and McLaughlin, 1996). It is not clear whether what occurs in *A. pulcherrimum* is unique within Basidiomycota and whether this species retains an ancestral division pattern.

To address these uncertainties, we identified yeast species within the Agaricostilbomycetidae (as described by Swann et al., 2001) through a molecular analysis of a range of urediniomycetous taxa. Two taxa, *Stilbum vulgare* and *Bensingtonia yuccicola*, were chosen based on their degree of relatedness to *A. pulcherrimum*. The monotypic, dimorphic *S. vulgare* produces a minute stipitate sporocarp with a spherical head, bear-

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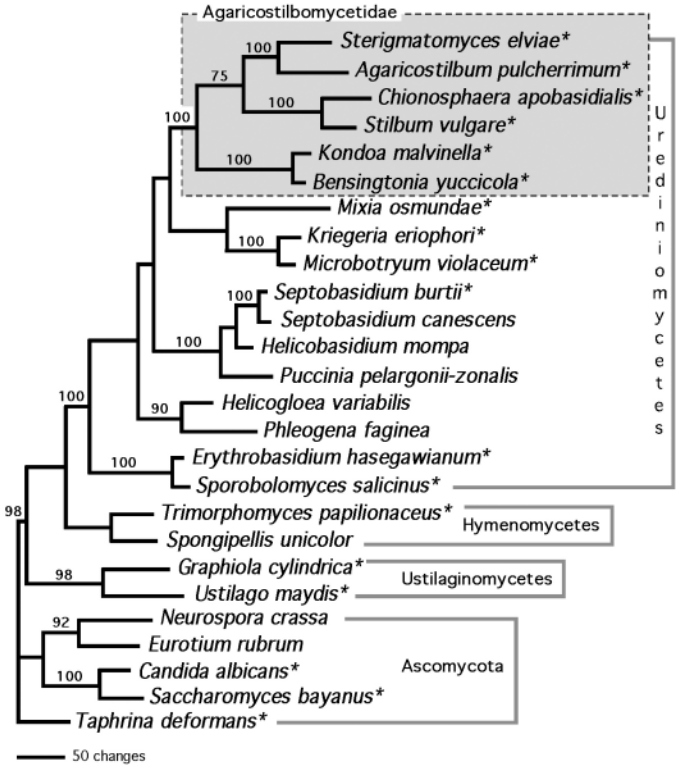


Fig. 1. One of two most parsimonous phylogenetic trees of mainly yeast-forming ascomycetes and basidiomycetes differing only in the topology of the ascomycetes. Taxa that are yeasts or have dimorphic life cycles with a yeast stage are indicated with an asterisk. The Agaricostilbomycetidae is strongly supported. Note the sibling relationship of *Chionosphaera apobasidialis* and *Stilbum vulgare* and that *S. vulgare* is more closely related to *Agaricostilbum pulcherrimum* than either is to *Bensingtonia yuccicola*. CI = 0.547; HI = 0.453; RI = 0.606; RC = 0.332.

ing two-celled basidia; each cell produces one basidiospore, suggesting binucleate basidiospores. Basidiospores germinate to form the yeast stage. This organism is widely distributed on a variety of substrates, such as angiosperm wood and bark, weathered and dead inflorescences, and mushrooms; it has been reported from Europe, North America, and Australia (Seifert et al., 1992). *Bensingtonia yuccicola* is a mitosporic yeast that produces ballistoconidia. It was reported from a dead fallen yucca leaf in Vancouver, British Columbia, Canada (Nakase and Suzuki, 1988).

In the following report, we present a molecular phylogeny for representatives of the Agaricostilbomycetidae of the Urediniomycetes and elucidate the mitotic and cytoskeletal patterns in the yeast phase of *S. vulgare* and *B. yuccicola*. The results are compared to those from *A. pulcherrimum* and other yeasts, and their phylogenetic relevance is discussed.

MATERIALS AND METHODS

Phylogenetic analysis—To determine phylogenetic relationships of *S. vulgare* and *B. yuccicola*, nuclear small subunit rRNA gene sequences were acquired for 25 species with an emphasis on yeast-forming taxa (Appendix; see Supplemental Data accompanying the online version of this article). The sequences for *Agaricostilbum pulcherrimum*, *Phleogena faginea*, *Septobasidium burtii*, *Septobasidium canescens*, *Sterigmatomyces elviae*, and *Stilbum vulgare* are newly published here. The outgroups were species of Ascomycota, Ustilaginomycetes, and Hymenomycetes. Species of Urediniomycetes were selected to represent the main clades in the class, as well as species related to *S. vulgare* and *B. yuccicola*. Other yeasts were selected for which microtubule localizations have been published. All species were grown and harvested as in Swann et al. (1999).

DNA was extracted using the method of Lee and Taylor (1990) and was amplified and purified as described in Swann et al. (1999). Sequences were obtained by cycle sequencing using fluorochrome-labeled dideoxynucleotides (ABI sequencing apparatus, Molecular Genetics Instrumentation Facility, University of Georgia, Athens, Georgia, USA). DNA sequence data were assembled and checked for accuracy by comparing forward and reverse strands. DNA sequences were aligned using the program CLUSTAL W (Thompson et al., 1994) and the multiple sequence editor in MacVector (version 7.0, Genetics Computer Group, Madison, Wisconsin, USA). DNA sequence alignment and trees have been submitted to TreeBASE (www.treebase.org): study accession number S1071, matrix accession number M1827.

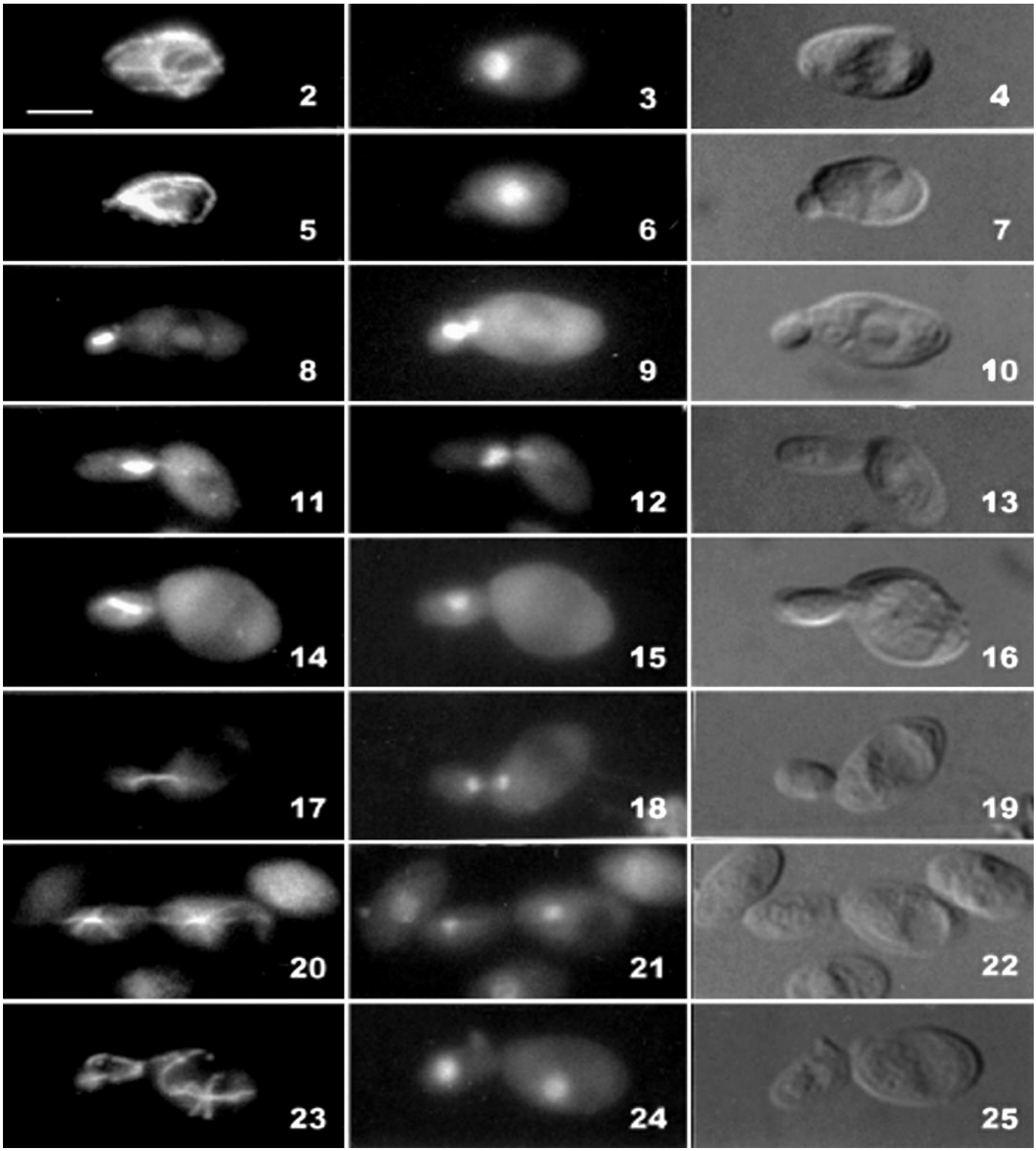
Unweighted and weighted phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford, 2003). Of 1843 characters, 493 were parsimony informative. Twenty-seven characters (190–199, 240–242, 679–685, 782–785, 1554–1556) were removed from the analysis, three sequence regions were eliminated because of questionable alignment, and two unique insertions in *Mixia* were reduced to single characters. Parsimony trees were obtained using a heuristic search, stepwise addition, random addition sequence, 10 replicates. Bootstrap support was determined with a full heuristic search, 1000 replicates. Base change frequencies were determined for the two parsimony trees using unambiguous changes in MacClade version 4.0 (Maddison and Maddison, 2000). Less frequent base changes were weighted more heavily than more frequent changes. Weights were determined as in Swann et al. (1999).

Cultures and cell preparation—*Bensingtonia yuccicola* strain ML 3193 (CBS 7331) was obtained from Jack Fell (Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, Key Biscayne, Florida, USA), and *Stilbum vulgare* strain RJB 75–9295-B was obtained from R. J. Bandoni (Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada). Cultures were maintained on malt yeast peptone (MYP) agar (Bandoni and Johri, 1972) and incubated at 21°C under fluorescent light under a 16-h light:8-h dark cycle.

A modification of the method of Taylor and Wells (1979) was used to synchronize budding to maximize the number of dividing cells. Twenty-four-hour-old cultures were used to inoculate new MYP plates, 0.75 mL of sterile distilled water was added, and yeasts were spread with a sterilized bent glass rod. For *Bensingtonia yuccicola*, budding was synchronized at 16 h and for *S. vulgare*, at 24 h.

Freeze-substitution and immunofluorescent labeling—Freezing, fixation, and immunolabeling procedures followed those outlined in Frieders and McLaughlin (1996) and McLaughlin et al. (1996), with slight modifications. Following collection on Mylar, plunge-freezing, and freeze-substitution in methanolic formaldehyde (3.7%), the cells were warmed gradually to room temperature. Cells were postfixed in a solution of 90% methanolic formaldehyde and 10% phosphate buffer containing 0.1 mol/L magnesium sulfate and 0.1 mol/L EGTA (both added to stabilize microtubules), followed by a secondary postfixation in 85% methanolic formaldehyde plus 1% glutaraldehyde in the same buffer. Yeast cells were rehydrated in an increasing buffer series. Some cells were released from the Mylar at each rehydration step; these cells were collected by centrifugation and rehydrated separately from those cells still attached to Mylar, until all cells were collected. Wall digestion, membrane permeabilization, and blocking reactions and subsequent rinses used phosphate-buffered saline with 0.1 mol/L magnesium sulfate and 0.1 mol/L EGTA.

Immunolocalization was performed using monoclonal antialpha-tubulin YOL1/34 (Amersham, Little Chalfont, UK) diluted 1:100 in buffered 1% bovine serum albumin. The secondary antibody was fluorescein isothiocyanate (FITC)-conjugated antirat IgG. Following labeling and staining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI), cells were mounted in Tris-HCl and 1,4-diazobicyclo(2,2,2)-octane in glycerol. Prepared slides were viewed



Figs. 2–25. Interphase and mitosis in *Bensingtonia yuccicola* yeast cells. Each row contains three views of the same cell: left, immunofluorescence-localized microtubules; center, DAPI-stained nuclei; right, DIC (see Materials and Methods: Freeze-substitution and immunofluorescent labeling). Bar = 5 μm. **2–4**. Interphase cell with microtubules lining the cell cortex. **5–7**. Prophase. Bud initiation prior to nuclear migration. Cortical microtubules in the bud and adjacent region of the parent. **8–10**. Early metaphase. Nucleus migrating into the bud where spindle is forming. **11–13**. Metaphase with nucleus mostly in the bud. **14–16**. Late metaphase with astral microtubules forming. **17–19**. Anaphase with spindle with more prominent astral microtubules elongating through the parent–bud junction. **20–22**. Telophase with prominent astral microtubules and spindle remnants. **23–25**. Interphase with cortical microtubules.

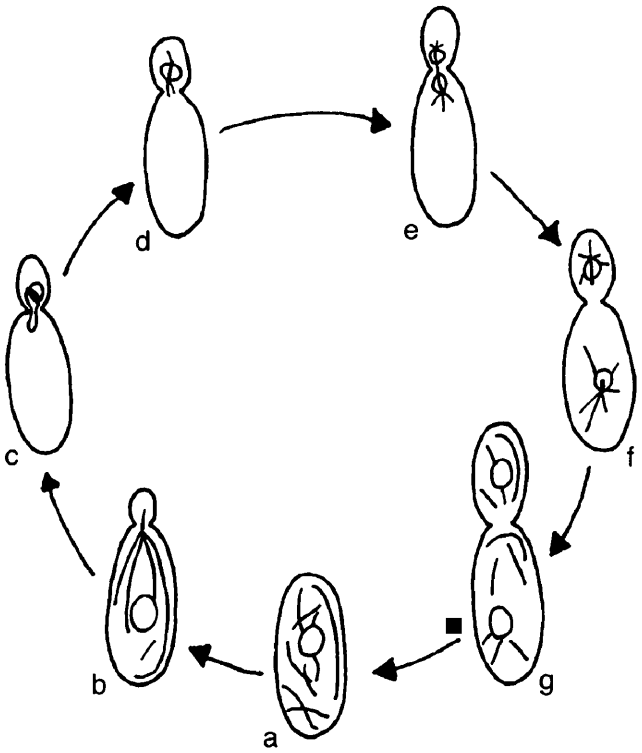


Fig. 26. Diagrammatic reconstruction of the microtubule cytoskeleton during the cell cycle in *Bensingtonia yuccicola* yeast cells; a, interphase; b, prophase; c, early metaphase; d, metaphase; e, anaphase; f, telophase; g, interphase.

with a Zeiss Axioskop using a Neofluor 100×/1.3 NA objective with an Optovar setting of 1.25× or 1.6×, barrier filters appropriate for FITC and DAPI observation, and differential interference contrast (DIC) optics. Photographs were taken using Kodak T-Max 400 ISO film at ISO 1600 (Kodak, Rochester, New York, USA).

RESULTS

Phylogenetic analysis—In the unweighted analysis, there were two most parsimonious trees, one of which is shown in Fig. 1. The two trees differed only in the topology within the Ascomycota. Weighting did not change the number of most parsimonious trees or the tree topologies (data not shown), but it did have a slight but nonsignificant effect on the bootstrap support values. However, when more taxa from the Agaricostilbomycetidae were included in the weighted analysis, bootstrap support value for the branch that links the *Agaricostilbum* + *Sterigmatomyces* and *Stilbum* + *Chionosphaera* clades was increased above 90 (not shown).

Bensingtonia yuccicola and *Kondoa malvinella* formed a strongly supported subclade, distinct from a subclade containing other members of the Agaricostilbomycetidae. Within the latter subclade, there were two well-supported lineages: *Sterigmatomyces elviae* and *Agaricostilbum pulcherrimum*; *Chionosphaera apobasidialis* and *Stilbum vulgare*. The close relationship between *C. apobasidialis* and *S. vulgare* is established for the first time. Thus, *B. yuccicola* is distantly related to the other organisms, *S. vulgare* and *A. pulcherrimum*, whose mitotic patterns are compared here.

Bensingtonia yuccicola mitosis—Cells of *Bensingtonia yuccicola* (Figs. 2–26) were generally ovoid in shape; however, some cells were nearly spherical, with buds forming from the parent in an apical to subapical position in all cells studied. All parent cells and most cells without buds contained a vacuole of varying size, clearly seen in DIC images (Figs. 4, 7, 10, 13, 16, 19, 22, 25). Occasionally, the vacuole interfered with the microtubule image (Fig. 20) but did not affect its interpretation.

Interphase cells (Figs. 2–4, 26a) possessed cytoplasmic microtubules of varying length and orientation, and many were cortical. The nucleus was approximately in the center of the cell and was of maximum size. A nucleolus was present at this stage (Fig. 3). Buds were initiated during prophase (Figs. 5–7, 26b) at which time microtubules appeared to be strictly cortical and were aligned parallel to the long axis of the cell, forming a basket-like structure focused at the parent–bud junction. As prophase progresses, the nucleus moved toward this junction (not illustrated).

At metaphase (Figs. 8–16, 26c, 26d), spindles were located in the bud oriented approximately parallel to the long axis of the bud. Bud size was variable and not closely correlated with the spindle length. Early in metaphase, most of the chromatin was located in the bud associated with the spindle, but some chromatin remained in the parent (Figs. 9, 12). It became entirely located within the bud as metaphase proceeded (Fig. 15). Spindle initiation was not observed. At early metaphase (Figs. 8–10, 26c), spindles were short and lacked astral microtubules. Later in metaphase (Figs. 11–16, 26d), the spindle elongated minimally, the chromatin became fully condensed, and short asters were observed.

The spindle began its major elongation during anaphase (Figs. 17–19, 26e), with a concomitant separation of chromatin. One spindle pole and the associated chromatin extended back into the parent. Astral microtubules became more pronounced at each pole. At telophase (Figs. 20–22, 26f), remnants of broken spindles were observed. Both asters reached their greatest development, extending to the cell periphery. Nuclei remained condensed at the spindle poles. At post-mitotic interphase (Figs. 23–25, 26g), asters disappeared and the cortical microtubule cytoskeleton gradually reformed in a pat-

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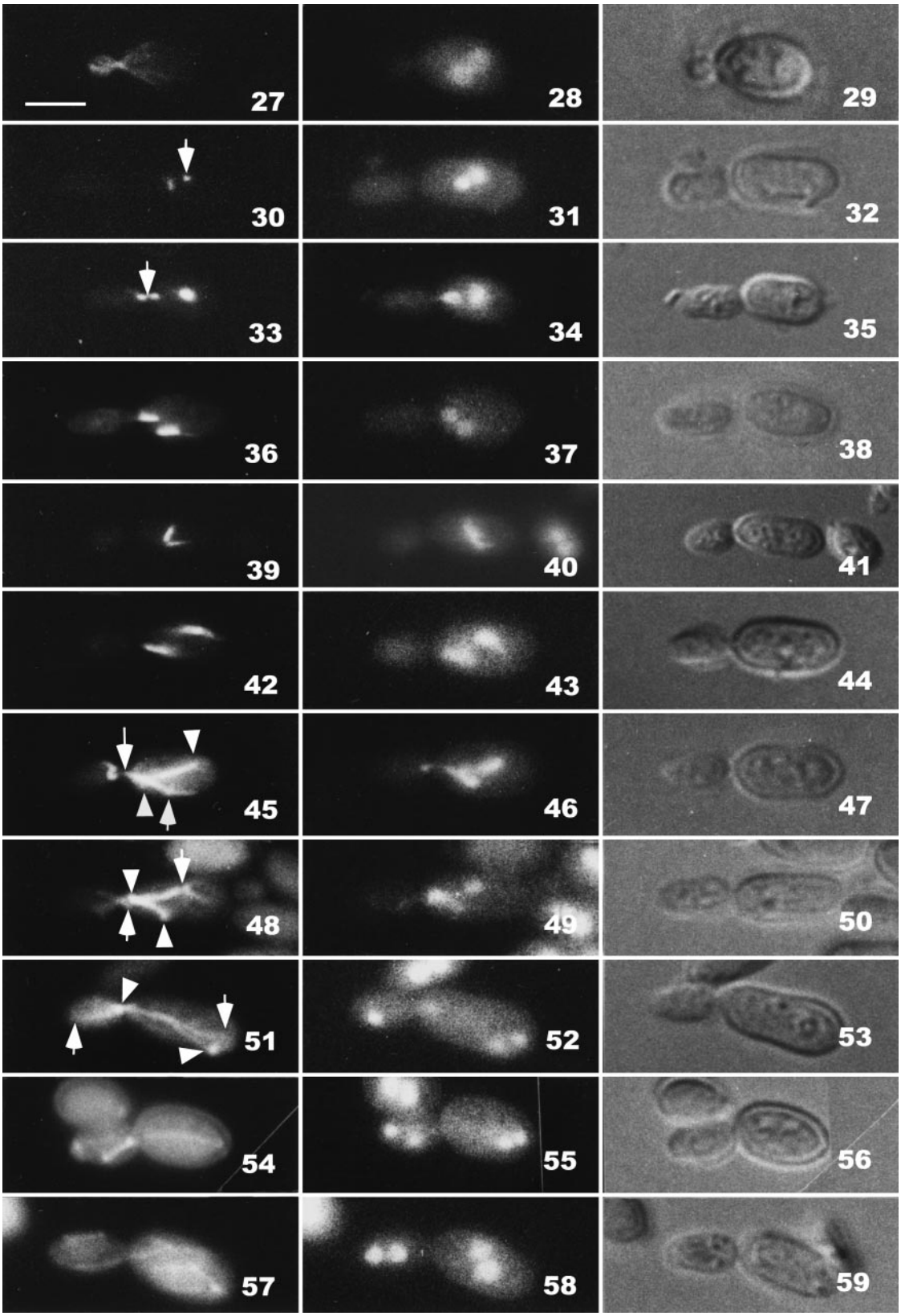
Figs. 27–59. Interphase and mitosis in *Stilbum vulgare* yeast cells. Each row contains three views of the same cell: left, immunofluorescence-localized microtubules; center, DAPI-stained nuclei; right, DIC. Bar = 5 μm. **27–29**. Prophase with cortical microtubules lining bud and adjacent parent. **30–32**. Prometaphase. Spindle poles unseparated in one nucleus (arrow) and separating in the other. **33–41**. Metaphase. Forming spindles with varying orientations within the parent and condensing chromatin. Arrow, spindle at parent–bud junction. **42–44**. Metaphase/anaphase. Chromatin arranged along the elongating spindles within the parent. **45–50**. Anaphase. **45–47**. One nucleus entering the bud preceded by astral microtubules with arrows marking the spindle poles. Arrowheads mark poles of second nucleus. **48–50**. Well-developed astral microtubules and chromatin at spindle poles (arrows) of one nucleus. Arrowheads mark poles of second nucleus. **51–53**. Anaphase and telophase. Arrowheads mark spindle poles of anaphase nucleus, and arrows mark poles of telophase nucleus. **54–56**. Telophase. Chromatin at spindle poles. Interphase cell adjacent to bud without visible cytoplasmic microtubules. **57–59**. Interphase. Bud and parent each binucleate.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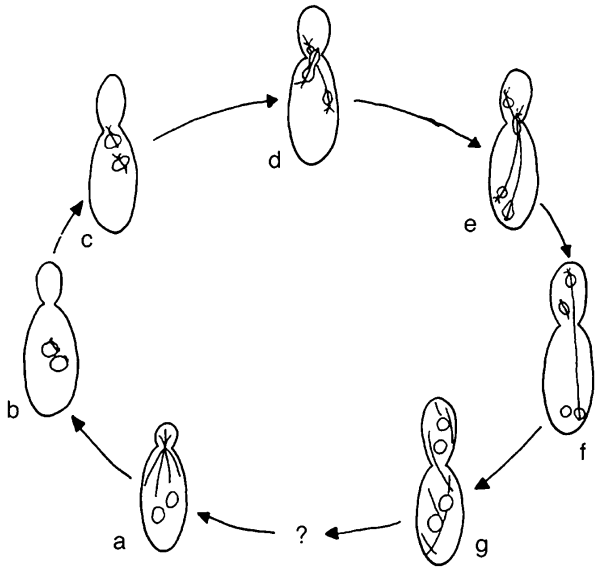

Fig. 60. Diagrammatic reconstruction of the microtubule cytoskeleton during the cell cycle in the binucleate yeast phase of *Stilbum vulgare*; a, prophase; b, prometaphase; c, metaphase; d, anaphase; e, anaphase; f, telophase; g, interphase.

tern similar to that seen in late interphase (Fig. 2). Daughter nuclei enlarged and appeared in random positions in both the parent and bud. Nucleoli reformed at this stage.

Stilbum vulgare mitosis—Cells of *Stilbum vulgare* (Figs. 27–60) were ovoid and budded apically. Almost all cells of this species were binucleate, but a few uninucleate cells were observed (not illustrated). Vacuoles were smaller than those in *B. yuccicola* and did not interfere with microtubule observation. Non-budding interphase cells were common, and the nuclei stained readily, but the microtubule cytoskeleton could not be localized despite many attempts (see upper cell in Figs. 54–56). With bud initiation, microtubule localization became possible (Fig. 27).

At prophase (Figs. 27–29, 60a), a cortical microtubule basket formed at, and focused on, the parent–bud junction, much like that in *B. yuccicola*. An array of microtubules extended from this focus point into the bud (Figs. 27, 60a). At this stage, the nuclei appeared to be uncondensed; they were located distally within the parent and not closely associated with the microtubule basket. Nucleoli were observed at this stage (not illustrated).

The two nuclei in each cell proceeded through mitosis asynchronously, and the spindles often were oriented at different angles. While the two mitotic apparatuses could be viewed clearly and interpreted via through-focusing, this clarity was not apparent in the two-dimensional photographic images.

Spindle initiation occurred in the parent (Fig. 30, arrow); early spindle elongation occurred primarily in the parent (Figs. 30, 33, 36) and rarely in the parent–bud junction (Fig. 33, arrow). During prometaphase (Figs. 30–32, 60b), the localized microtubules formed around the spindle pole body and appeared as a small bilobed dot associated with the condensing chromatin. At early metaphase (Figs. 30–35, 60b), the spindle elongated somewhat and was oriented perpendicular to the long axis of the cell. Chromatin was somewhat more condensed. Nuclei no longer were located distally in the parent and varied from a central to proximal position in the parent. As metaphase proceeded (Figs. 36–41, 60c), spindles were oriented obliquely or nearly parallel to the long axis of the cell. Short and sparse astral microtubules appeared, and chromatin became progressively more condensed until it resembled a metaphase plate. At meta-anaphase (Figs. 42–44) the asters became somewhat more pronounced and the chromatin began to move toward the poles.

In anaphase (Figs. 45–53, 60d, 60e), the spindle elongated completely, chromatin separated and reached the spindle poles, and astral microtubules developed fully. The proximal spindle poles and associated chromatin entered the bud one at a time, doing so at different anaphase stages, sometimes entering at early anaphase (Figs. 45–47, upper arrow), mid-anaphase (Fig. 48, lower arrow), or at late anaphase (Figs. 51–53, upper arrowhead). In early to mid-anaphase (Figs. 45, 48), there was limited spindle elongation, the distal spindle poles remained relatively stationary, while the proximal poles moved toward and sometimes through the parent–bud junction. The spindle elongated greatly during late anaphase (Figs. 51–53, 60e). By mid-anaphase (Figs. 49, 60d), the daughter chromatin separated fully and reached the poles. As anaphase proceeded, astral microtubules reached their maximum length, extending to or nearly to the cell periphery (Fig. 48).

In telophase (Figs. 51–56, 60e, 60f), spindles were fully elongated, extending from the apex of the bud to the distal end of the parent. By late telophase, spindles were broken and partially disassembled (Fig. 54). Astral microtubules were short, forming a minute fan at the poles. The forming daughter nuclei, located at the cell extremities, were still condensed (Fig. 55). In postmitotic interphase (Figs. 57–59, 60g), long, randomly oriented, cortical cytoskeletal microtubules were present. Nuclei were fully decondensed and medianly positioned, and nucleoli appeared (Fig. 58).

DISCUSSION

The molecular data presented in our analysis corroborate similar studies of taxa related to *Agaricostilbum*. Our tree topology, based on the nuclear small subunit gene, agrees with those of Scorzetti et al. (2002), which used partial nuclear large subunit gene and ITS regions, and we obtained much higher bootstrap support values. These results place *Chionosphaera* in the Agaricostilbomycetidae, as do other studies on this subclass (Swann et al., 1999, 2001; Fell et al., 2001; Scorzetti et al., 2002), and establish it as a sibling taxon to *Stilbum vulgare*. Based on structural characters, *S. vulgare* has been classified within the Chionosphaeraceae (Oberwinkler and Bauer, 1989), and molecular evidence supports this placement. Although *S. vulgare* was included in the Atractiellales (Oberwinkler and Bandoni, 1982), our study further confirms its removal from this order (Oberwinkler and Bauer, 1989).

Previous studies of mitosis using immunofluorescence methods in three genera of basidiomycetous yeasts, each in a different subclass of Urediniomycetes (Frieders and McLaughlin, 1996; McLaughlin et al., 1996), have demonstrated that the typical mitotic pattern involves nuclear division in the bud rather than the parent and are in agreement with ultrastructural studies of basidiomycetes in general (McCully and Robinow, 1972a, b; Poon and Day, 1976a, b; Taylor and Wells, 1979; Boekhout and Linnemans, 1982; Mochizuki et al., 1987). The present study focused on three genera in a single subclass and shows that mitotic studies using immunofluores-

cence localizations have phylogenetic significance. *Agaricostilbum pulcherrimum* (Frieders and McLaughlin, 1996) and *Stilbum vulgare* have cystoskeletal and mitotic characters suggestive of an intermediate position between ascomycetes and basidiomycetes. In both species, spindle initiation and early elongation occur in the parent, as in ascomycetes (Kilmartin and Adams, 1984; Barton and Gull, 1988; Danková et al., 1988); early in mitosis, nuclear migration toward the bud is associated with a cortical basket of microtubules and late in mitosis, the spindle in the bud elongates back into the parent, as in other basidiomycetes (Frieders and McLaughlin, 1996; McLaughlin et al., 1996). The mitotic pattern in budding cells of *Bensingtonia yuccicola* follows that of typical basidiomycetes with spindle initiation originating in the bud. If common equals primitive, then these results can be interpreted as indicating that the ascomycetous pattern in *A. pulcherrimum* and *S. vulgare* is derived rather than basal. Also, these two taxa are gasteroid, a condition known to be derived multiple times within Basidiomycota. Correctly interpreting character evolution, however, requires molecular phylogenies from multiple genes presently being pursued through the Assembling the Fungal Tree of Life project (http://ocid.NACSE.ORG/research/aftol/), because the deep roots for basidiomycetes, as for fungi in general, are unknown.

In dimorphic basidiomycetes, production of dikaryotic asexual propagules is common, although these generally arise from the dikaryotic mycelium rather than from the yeast stage (Bandoni, 1995). Seifert et al. (1992) reported the yeast stage of *S. vulgare* to be haploid. Although some monokaryotic yeast cells were found during our investigations of *S. vulgare*, the vast majority were binucleate and regenerated via a standard budding method. The tremellaceous *Trimorphomyces papilionaceus* also has a dikaryotic yeast stage, but its method of regeneration differs from that of *S. vulgare* yeasts in that the parent cell produces two haploid conidia that fuse and enlarge to become a dikaryotic yeast cell (Oberwinkler and Bandoni, 1983). Less than 1% of known yeasts have been studied using these immunofluorescence cytological techniques. Of those studied, *S. vulgare* is the only dikaryotic species examined. The dikaryotic yeast stage of *S. vulgare* is unusual and may be a by-product of its two-celled basidium. It is unclear whether the binucleate condition influences the site of mitosis. In *A. pulcherrimum* the uninucleate yeast stage also initiates mitosis in the parent. There is no obvious reason why the binucleate condition would require initiation of nuclear division in the parent. To address the apparent uniqueness of the mitotic pattern in *S. vulgare*, how widespread the dikaryotic condition is among basidiomycetous yeasts would have to be determined.

To date five genera of basidiomycetous yeasts have been studied with immunofluorescence methods, and the phylogenetic utility of cytoskeletal and mitotic characters is supported. Based on these limited studies, there appear to be major mitotic differences between ascomycetous and basidiomycetous yeasts. Characters that unite the basidiomycetes thus far are the cortical basket of microtubules early in mitosis associated with nuclear migration toward the bud, and spindle elongation from the bud back into the parent. As in basidiomycetes, yeasts are scattered through all major ascomycete clades (Kurtzman and Sugiyama, 2001). The mitotic patterns of even fewer ascomycetous, than basidiomycetous, yeasts have been analyzed, and these studies are not representative of ascomycetous yeast taxonomic diversity. We anticipate that further cytological studies of yeasts in both phyla will yield additional characters of phylogenetic value.

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